

## STRUCTURAL STUDIES OF A NEW HEMOGLOBIN: HbJ Lens, $\beta 13(\text{A}10) \text{Ala} \rightarrow \text{Asp}$

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### 1. Introduction

This new variant was found in a 2 year old French child, obese and unable to walk. Many investigations were done and during hematological examination, a very mild microcytic hypochromic anemia was found. A fast-moving hemoglobin was noted on cellulose acetate strip electrophoresis. It migrated as HbH or HbJ. This abnormal hemoglobin was characterized by structural studies as  $\alpha_2\beta_2 13(\text{A}10)\text{Ala} \rightarrow \text{Asp}$  and was named HbJ Lens.

### 2. Methods

#### 2.1. Routine investigation

Blood was collected in heparin and hemolysate prepared by routine procedures. Electrophoresis was done on cellulose-acetate in 0.058 M Tris–0.15 M glycine buffer (pH 9.5). HbA<sub>2</sub> level was estimated by DEAE-cellulose chromatography in [1]. Isopropanol solubility test was carried out according to [2] and the heat stability test as in [3]. Other hematological studies were performed according to standard methods.

#### 2.2. Structural studies

Globin was prepared by the acid acetone precipitation method at  $-20^{\circ}\text{C}$  and globin chain electrophoresis was performed in veronal 6 M urea buffer (pH 8.0) as in [4]. The pure abnormal  $\beta$ -chain was obtained by chromatography on carboxymethylcellulose column in 8 M urea according to [5]. After gel filtration on a column (150  $\times$  2.5 cm) of Sephadex G-25 (coarse) in 0.2 M aqueous acetic acid, the  $\alpha$ - and  $\beta$ -chains were freeze dried. The abnormal  $\beta$ -chain was *S*-aminoethylated according to [6] and then desalted on a column of Sephadex G-25. Analytical paper fingerprints of a tryptic digest of aminoethylated  $\beta$ -chain were prepared

at pH 6.4 according to [7]. The abnormal tryptic peptide was eluted from preparative paper fingerprints with 6 N HCl after previous purification by paper electrophoresis at pH 3.5 and subjected to acid hydrolysis during 24 h. Amino acids analyses were carried out on a Biotronik LC 2000 amino acid analyzer. The same abnormal peptides stained by fluorescamine were eluted from other preparative paper fingerprints by 8% (v/v) aqueous acetic acid. The sequential analysis of amino acid residues was performed by Edman's manual degradation modified as in [8]. Phenyl thiohydantoin amino acid derivatives were identified by thin-layer chromatography in the solvent system of [9] and by HPLC on a C18  $\mu$ -Bondapak column as in [10].

### 3. Results and discussion

#### 3.1. Hematological data for the HbJ Lens

The hematological investigation gave the following results: Hb, 13.8 g/dl; PCV, 0.42 l/l; RBC,  $5.49 \times 10^{12}/\text{l}$ ; WBC,  $9.5 \times 10^9/\text{l}$ ; MCV, 73 fl; MCHC, 35 g/dl; MCH, 25 pg; serum iron, 69 g/dl; platelets,  $230 \times 10^9/\text{l}$ .

#### 3.2. Electrophoretic studies and stability test

Electrophoresis of the hemolysate at alkaline pH on cellulose acetate revealed the presence of an abnormal component which migrated more anodically than HbA in a proportion of  $\sim 53.3\%$ , probably increased by the presence of HbA<sub>3</sub>. This component was characterized as HbJ (fig.1). The proportion of HbA<sub>2</sub> was  $\sim 3\%$  and HbA 43.7%. No abnormality was found by isopropanol or heat stability tests. Globin chain electrophoresis in 6 M urea, however indicated the presence of an abnormal  $\beta$ J chain migrating more anodically than normal  $\beta$ -chain.

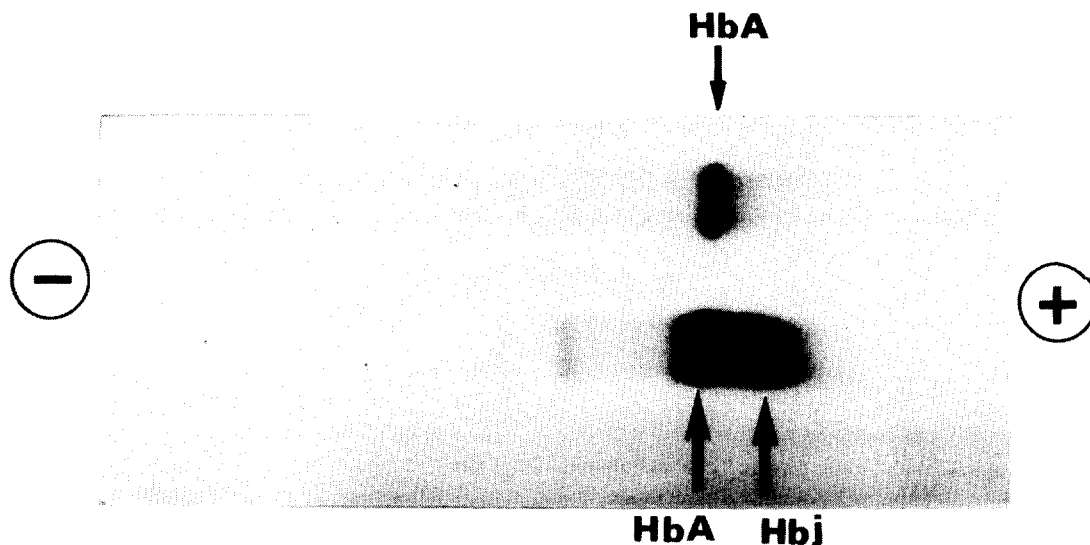


Fig.1. Cellulose acetate electrophoresis of HbJ and HbA hemoglobins in 0.058 M Tris–0.15 M glycine buffer (pH 9.5) as in section 2.

### 3.3. Structural studies

The fingerprints of the abnormal  $\beta$ -chain tryptic peptides showed that the  $\beta^A\text{TpII}$  (fig.2) which consists of residues 9–17 of the  $\beta$ -chain was absent and stained negatively for tryptophan and very poorly with ninhydrin. We have also found 2 tryptophan-positive peptides: peptide (a) overlapping  $\beta^A\text{TpIX}$  in the neutral region and peptide (b) between  $\beta^A\text{TpIII}$  and  $\beta^A\text{TpXI}$ . Peptide (b) also stained positively for argi-

nine. The amino acid composition of the peptide (a) recovered the whole sequence of the peptide  $\beta^A\text{TpII}$  except that one alanine was replaced by an extra residue of aspartic acid (table 1). The amino acid composition of peptide (b) suggested that this peptide was an abnormal  $\beta\text{TpII–III}$  peptide with addition of one aspartic acid and the absence of an alanine. Since there are two residues of alanine in  $\beta^A\text{TpII}$  at position  $\beta 10$  and  $\beta 13$ , we had to perform the sequence studies and

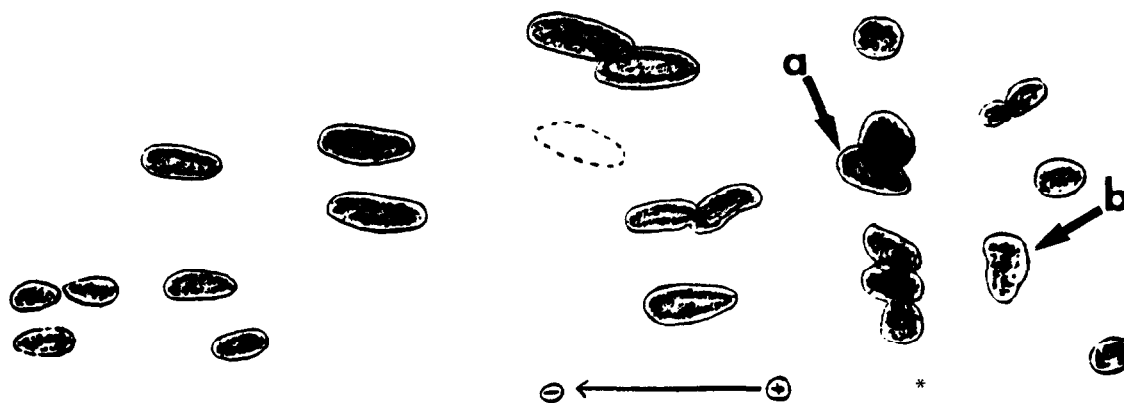


Fig.2. Fingerprints of the tryptic peptides of the amino-ethylated  $\beta$ -chain of HbJ. Electrophoresis at pH 6.4 (60 V/cm, 1 h). Ascending chromatography in the upper phase of pyridine–isoamyl alcohol–water (600:600:700, by vol.) for 20 h. Peptides were located by staining with ninhydrin. (\*) Origin (a) position of abnormal  $\beta\text{TpII}$  (b) position of abnormal  $\beta\text{TpII–III}$ . The broken line indicates the normal position  $\beta^A\text{TpII}$  of which traces remained on the fingerprint.

Table 1  
Amino acid composition of peptide TpII from HbJ Lens

Amino acid	HbJ Lens	Expected for HbA
Asp	0.7	0
Thr	1.0	1
Ser	1.2	1
Gly	1.2	1
Ala	1.2	2
Val	0.8	1
Leu	0.9	1
Lys	0.9	1
Trp <sup>a</sup>	+	+

<sup>a</sup> Tryptophan was identified by UV lamp and by its specific reaction

the following sequence of peptide (a) was found:

Ser—Ala—Val—Thr—Asp—(Leu, Trp, Gly, Lys)  
9 10 11 12 13 14 15 16 17

instead of:

Ser—Ala—Val—Thr—Ala—Leu—Trp—Gly—Lys

in the normal peptide. These findings indicated that peptide (a) was the tryptic peptide  $\beta$ TpII in which substitution had occurred at position  $\beta$ 13(A10). No hemoglobin with the substitution  $\beta$ 13(A10)Ala  $\rightarrow$  Asp has been described before. So we named this abnormal hemoglobin after the city where it was discovered.

#### 4. Discussion

Sequence studies have shown that in  $\beta^J$ TpII, alanine at position  $\beta$ 13 has been replaced by an aspartic acid residue. This substitution increased the negative charge of the tryptic peptide  $\beta^J$ TpII and explained its migration towards the anode. The presence on the fingerprint of an abnormal  $\beta^J$ TpII—III peptide indicated that trypsin was not able to split completely the pep-

tide bond between lysine  $\beta$ 17 and valine  $\beta$ 18. It is not impossible to think that a carboxyl group of aspartic acid at position  $\beta$ 13 should be capable of forming a salt bridge with the  $\epsilon$  side chain amino group of lysine  $\beta$ 17 and therefore make trypsin unable to completely split the protein on the C-terminal side of lysine  $\beta$ 17. The substitution in HbJ Lens is at an external site which therefore plays no part in subunit contacts or haem binding. Such an external substitution probably does not affect the properties of hemoglobin tetramer, and the mild microcytic anemia is also probably due to iron deficiency.

#### Acknowledgements

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